THE PREVALENCE, THE RESISTANCE PATTERN, THE RISK FACTORS AND THE CLINICAL PROFILE OF MULTI DRUG RESISTANT TYPHOID FEVER (MDRTF) AND NALIDIXIC ACID RESISTANT SALMONELLA TYPHI (NARST) AMONG CULTURE POSITIVE TYPHOID FEVER IN HOSPITALIZED CHILDREN LESS THAN 12 YEARS OF AGE

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CERTIFICATE

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DECLARATION

I declare that this dissertation entitled "**The prevalence, the resistance pattern, the risk factors and the clinical profile of multi drug resistant typhoid fever (MDRTF) and nalidixic acid resistant Salmonella typhi (NARST) among culture positive typhoid fever in hospitalized children less than 12 years of age** " has been conducted by me at the Institute of Child Health and Hospital for Children. It is submitted in part of fulfillment of the award of the degree of M.D. (Pediatrics) for the March 2007 examination to be held under the Tamil Nadu Dr. M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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CONTENTS

INTRODUCTION

Typhoid history

Typhoid fever or enteric fever is an age-old disease known for centuries. Patients may become delirious, the typhoid state for which the disease is named (typhus from the Greek, stupor). Its symptoms and epidemiology overlap with that of typhus (camp fever), so typhoid has been confused with this rickettsial disease for much of its history. The name typhoid implies a "typhus-like" condition, and these two disease have been clearly delineated only since the mid-nineteenth century. In England, Huxham (1739) in his "Essay on Fevers" described the Plymouth epidemic of 1737 and he distinguished between putrid typhus (febris putrida) and described nervous fever (febris nervosa lenta) or what is now recognized as typhoid. By the nineteenth century, typhoid and typhus were recognized as disease with distinct clinical features. The great Parisan clinician Pierre – Charles –Alexandre Louis named the condition fever typhoid in his major work on the disease in 1829. Carl Joseph Eberth described the typhoid organism in the issue of patients in 1880 and George Galfky isolated and grew this organism in 1884. Friz Kauffmann (1937) and Philip B. White (1926) studied the antigenic structures of the typhoid and typhoid –like bacteria in detail and the Kauffmann – White system classification became standard. This system classification of the genus Salmonella employs the H, O, and Vi antigenic specificities to identify a particular isolate.

Magnitude of the problem

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is

confused with those of many other febrile infections. On the basis of the literature [1,2] and the incidence of typhoid fever recorded in control groups in large vaccine field trials with good laboratory support it has been estimated that approximately 17 million cases of typhoid fever and 600 000 associated deaths occur annually [3]. The ratio of disease caused by S. typhi to that caused by S. paratyphi is about 10 to 1 in most of the countries where this matter has been studied. In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. Nevertheless, clinically apparent bacteraemic S. typhi infection in children aged under three years has been described in Bangladesh, India, Jordan, Nigeria, and elsewhere. Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increasing with age and being greater in females than in males.

The organism

Typhoid fever is caused by Salmonella typhi, a Gram-negative bacterium. A very similar but often less severe disease is caused by Salmonella serotype paratyphi A. S. typhi can be identified in the laboratory by several biochemical and serological tests. One of the most specific serologic tests is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated S. typhi and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (Citrobacter freundii, Salmonella paratyphi C and Salmonella dublin) but not in exactly the same genetic context.

Colony characteristics

Blood agar

On blood agar, S. typhi and S. paratyphi usually produce non-hemolytic smooth white colonies.

MacConkey agar

On MacConkey agar, salmonellae produce lactose non-fermenting smooth colonies.

Salmonella – Shigella (SS)agar

On SS agar, salmonellae usually produce lactose non-fermenting colonies with black centres (except S. paratyphi A, whose colonies do not have black centres).

Desoxycholate agar

On desoxycholate agar, salmonellae produce lactose non-fermenting colonies with black centres (except S. paratyphi A, whose colonies do not have black centres).

Xylose-lysine-desoxycholate agar

On xylose-desoxycholate agar, salmonellae produce transparent red colonies with black centres (except S. paratyphi A, whose colonies do not have black centres).

Hektoen enteric agar

On hektoen enteric agar, salmonellae produce transparent green colonies with black centres (except S. paratyphi A, whose colonies do not have black centres).

Bismuth sulfite agar

On this medium, salmonellae produce black colonies.

Biochemical identification

Suspected colonies obtained on the above media are screened by means of the following media/tests:

Organism	Kliger's iron agar				Motility	Indole	Urea	Citrate
	Slant	Butt	H_2S	Gas				
S. typhi	Alk	Acid	Wk+	-	+	-	-	-
S. paratyphi A	Ald	Acid	-	+	+	-	-	-
Other Salmonella	Alk	Acid	V	V	+	_	_	V
E. coli	Acid	Acid	-	+	+	+	-	-
Klebsiella spp.	Acid	Acid	_	++	_	V	+	+
Citrobacter spp.	V	Acid	+++	+	+	V	-	+
Proteus spp.	Alk	Acid	+	+	+	V	++	V

The production of acid turns the agar yellow. For the slant this denotes lactose fermentation and for the butt this denotes glucose fermentation.

Alk = alkaline, Wk = weak, V= variable result.

Serological identification of Salmonella

Salmonellae can be characterized by their somatic (O) and flagellar (H) antigens, the latter existing in some serotypes in phases 1 and 2. Some salmonellae also have an envelop antigen called Vi (virulence). The O antigen is usually determined by means of the slide agglutination test with group-specific antiserum followed by agglutination with factor antiserum. H antigen is usually determined by means of the tube agglutination test.

Transmission

Humans are the only natural host and reservoir. The infection is transmitted by ingestion of food or water contaminated with faeces. Ice cream is recognized as a significant risk factor for the

transmission of typhoid fever. Shellfish taken from contaminated water and raw fruit and vegetables fertilized with sewage, have been sources of past outbreaks. The highest incidence occurs where water supply serving large populations is contaminated with faeces. Epidemiological data suggest that waterborne transmission of S. typhi usually involves small inocula, whereas food borne transmission is associated with large inocula and high attack rates over short periods. The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period. In volunteers who ingested 10^9 and 10^8 pathogenic S. typhi in 45 ml of skimmed milk, clinical illness appeared in 98% and 89% respectively. Doses of 10^5 caused typhoid fever in 28% to 55% of volunteers, whereas none of 14 persons who ingested 10^3 organisms developed clinical illness. Although it is widely believed that Salmonella is transmitted via the oral route, the transmission of S. typhimurium via the respiratory route has been demonstrated in a mouse model.

Family studies were conducted in Santiago, Chile, during an era of high typhoid endemicity in order to ascertain whether chronic carriers were significantly more frequent in households where there were index cases of children with typhoid fever than in matched control households. . It was concluded that chronic carriers in households did not play an important role in transmission. In developed countries, on the other hand, typhoid is transmitted when chronic carriers contaminate food as a consequence of unsatisfactory food-related hygiene practices.

Pathogenesis

During an acute infection, S. typhi multiplies in mononuclear phagocytic cells before being released into the bloodstream. After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either

microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages that ingest the bacilli but do not generally kill them. Some bacilli remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by macrophages. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors. Incubation periods ranging from 3 days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (~1—10 bacteria per ml of blood).

Clinical features

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications. Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculum ingested, host factors (e.g. HLA type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid. Patients who are infected with HIV are at significantly increased

risk of clinical infection with S. typhi and S. paratyphi [4]. Evidence of Helicobacter pylori infection also represents an increased risk of acquiring typhoid fever.

•Acute non-complicated disease: Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever, up to 25% of patients show exanthem (rose spots) on the chest, abdomen and back.

•Complicated disease: Acute typhoid fever may be severe. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients and up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases. Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen.

Altered mental status in typhoid patients has been associated with a high case-fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic

symptoms, although rare, have been reported. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uraemic syndrome. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic carrier state may develop.

Diagnosis of typhoid fever

The definitive diagnosis of typhoid fever depends on the isolation of S. typhi from blood, bone marrow or a specific anatomical lesion. The presence of clinical symptoms characteristic of typhoid fever or the detection of a specific antibody response is suggestive of typhoid fever but not definitive. Blood culture is the mainstay of the diagnosis of this disease.

Although ox bile medium (Oxgall) is recommended for enteric fever pathogens (S. typhi and S. paratyphi), only these pathogens can be grown on it. In a general diagnostic laboratory, therefore where other pathogens are suspected, a general blood culture medium should be used. More than 80% of patients with typhoid fever have the causative organism in their blood. A failure to isolate the organism may be caused by several factors: (i) the limitations of laboratory media [5]; (ii) the presence of antibiotics [6]; (iii) the volume of the specimen cultured [7]; or (iv) the time of collection, patients with a history of fever for 7 to 10 days being more likely than others to have a positive blood culture. Bone marrow aspirate culture is the gold standard for the diagnosis of typhoid fever [8, 9, 10] and is particularly valuable for patients who have been previously treated, who

have a long history of illness and for whom there has been a negative blood culture with the recommended volume of blood. Duodenal aspirate culture has also proved highly satisfactory as a diagnostic test but has not found widespread acceptance because of poor tolerance of duodenal aspiration, particularly in children.

If a bacteriology laboratory is not available on site, clinical specimens for culture can be transported to a main laboratory for processing. For blood culture it is essential to inoculate media at the time of drawing blood. For other specimens it is advisable to make the time of transportation to the laboratory as short as possible. It is more important to process the specimens quickly than to keep them cold. Once they have been inoculated, blood culture bottles should not be kept cold. They should be incubated at 37°C or, in tropical countries, left at room temperature, before being processed in the laboratory.

The volume of blood cultured is one of the most important factors in the isolation of S. typhi from typhoid patients, 10-15 ml should be taken from schoolchildren and adults in order to achieve optimal isolation rates; 2-4 ml are required from toddlers and preschool children [7]. This is because children have higher levels of bacteraemia than adults. In some regions it may be impossible to collect such large volumes of blood and so alternative diagnostic methods may be necessary for cases in which blood cultures are negative. Because reducing the blood volume reduces the sensitivity of the blood culture, however an effort should be made to draw sufficient blood if at all possible. Blood should be drawn by means of a sterile technique of venous puncture and should be inoculated immediately into a blood culture bottle with the syringe that has been used for collection.

The optimum ratio of the volume of blood to traditional culture broth should be 1 to 10 or more (e.g. 1:12). In general, if 5 ml of blood are drawn they should be inoculated into 45 ml or more of broth. If 10-15 ml of blood are drawn the specimen can be divided into equal aliquots and inoculated into two or more blood culture bottles. This allows the use of standard blood culture bottles of 50 ml. For small children the volume of blood drawn can be reduced but should still be inoculated into 45 ml of culture broth. The blood culture bottle should then be transported to the main laboratory at ambient temperature (15°C to 40°C) as indicated above. Blood cultures should not be stored or transported at low temperatures. If the ambient temperature is below 15°C it is advisable to transport blood cultures in an incubator. In the laboratory, blood culture bottles should be incubated at 37°C and checked for turbidity, gas formation and other evidence of growth after 1, 2, 3 and 7 days. For days 1, 2 and 3, only bottles showing signs of positive growth are cultured on agar plates. On day 7 all bottles should be subcultured before being discarded as negative.

A typical blood culture bottle contains 45 ml of tryptic soy broth or brain heart infusion broth. These are inoculated with 5 ml of fresh blood and incubated at 37°C. Negatives should be kept for at least seven days. Because S. typhi is not the only bacterial pathogen found in blood, subculturing is performed on days 1, 2, 3 and 7 on non-selective agar. The best agar is blood agar (horse or sheep blood) as this allows the growth of most bacterial pathogens. If blood agar is not available, nutrient agar can be used in combination with MacConkey agar. In some laboratories the use of MacConkey agar alone is preferred as this allows the growth of only bile-tolerant bacteria such as S. typhi and does not allow the growth of many gram-positive contaminants. The contamination of blood cultures reduces isolation rates for S. typhi and should be prevented as far as possible. For suspected typhoid fever, subculture plates should be incubated at 37°C for

18-24 hours in an aerobic incubator.

The identification of colonies as S. typhi is straightforward if reagents of satisfactory quality are available. Colonies from solid media can be used for agglutination with specific antisera. Several salmonellae may share the same antigenic structure. Consequently, confirmation by means of biochemical tests is always necessary.

Antimicrobial susceptibility test for typhoid fever organisms

Antimicrobial susceptibility testing is crucial for the guidance of clinical management. Isolates from many parts of the world are now multidrug-resistant (MDR). Isolates are usually resistant to ampicillin, chloramphenicol, sulfonamide, trimethoprim, streptomycin and tetracycline. Alternative drugs that are used for treatment include: fluoroquinolones (e.g. ciprofloxacin), third-generation cephalosporins (e.g. ceftriaxone, cefotaxime), a monobactum beta-lactam (aztreonam) and a macrolide (azithromycin). Even though resistance to the first two has been noted they nevertheless remain useful [11]. Reduced susceptibility to fluoroquinolones is indicated by in vitro resistance to nalidixic acid [12].

Felix-Widal test

This test measures agglutinating antibody levels against O and H antigens. The levels are measured by using doubling dilutions of sera in large test tubes. Usually O antibodies appear on days 6-8 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on an acute serum (at first contact with the patient). A convalescent serum should preferably also be collected so that paired titrations can be performed. In practice, however, this is often difficult. At least 1 ml of blood should be collected each time in order to have a sufficient amount of serum. In exceptional circumstances the test can be performed on plasma without any adverse effect on the result.

The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has

blunted the antibody response. On the other hand, S. typhi shares O and H antigens with other Salmonella serotypes and has cross-reacting epitopes with other Enterobacteriacae, and this can lead to false-positive results. Such results may also occur in other clinical conditions, e.g. malaria, typhus, bacteraemia caused by other organisms, and cirrhosis. In areas of endemicity there is often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas.

It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which the antibody titre is considered significant. This is particularly important if, as usually happens, a single acute sample is available for testing. If paired sera are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic. Quality control of the test is achieved by running a standard serum with a known antibody titre in parallel in each batch of assays. The variations in the standard serum should not exceed one tube, i.e. double dilution.

Despite these limitations the test may be useful, particularly in areas that cannot afford the more expensive diagnostic methods. This is acceptable so long as the results are interpreted with care in accordance with appropriate local cut-off values for the determination of positivity. This test is unnecessary if the diagnosis has already been confirmed by the isolation of S. typhi from a sterile site.

IDL Tubex® test

The Tubex® test is simple (essentially a one-step test) and rapid (taking approximately two minutes). It exploits the simplicity and user-friendliness of the Widal and the slide latex

agglutination tests but uses the separation of coloured particles in solution to improve resolution and sensitivity. Specificity is improved by means of an inhibition assay format and by detecting antibodies to a single antigen in S. typhi only. A positive result given by Tubex® invariably suggests a Salmonella infection. Infections caused by other serotypes including S. paratyphi A give negative results. Tubex® has not been evaluated extensively but several trials are being planned. In a preliminary study involving stored sera the test performed better than the Widal test in both sensitivity and specificity [13].

Typhidot® test

This test makes use of the 50 kD antigen to detect specific IgM and IgG antibodies to S. typhi. It has undergone full-scale multinational clinical evaluation of its diagnostic value. This dot EIA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In order to increase diagnostic accuracy the original Typhidot® test was modified by inactivating total IgG in the serum sample. Studies with the modified test, Typhidot-M®, have shown that inactivation of IgG removes competitive binding and allow access of the antigen to the specific IgM when it is present. The detection of specific IgM within three hours suggests acute typhoid infection. Evaluations of Typhidot® and Typhidot-M® in clinical settings showed that they performed better than the Widal test and the culture method [14].

In laboratory diagnoses of typhoid fever the method used as the gold standard should approach 100% in sensitivity, specificity and positive and negative predictive values. Evaluation

studies have shown that Typhidot-M® is superior to the culture method. Although culture remains the gold standard it cannot match Typhidot-M® in sensitivity (>93%), negative predictive value and speed. Typhidot-M® can replace the Widal test when used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The high negative predictive value of the test suggests that Typhidot-M® would be useful in areas of high endemicity.

IgM dipstick test

The typhoid IgM dipstick assay is designed for the serodiagnosis of typhoid fever through the detection of S. typhi-specific IgM antibodies in serum or whole blood samples. The dipstick test provides a rapid and simple alternative for the diagnosis of typhoid fever, particularly in situations where culture facilities are not available. The assay can be performed by people without formal training and in the absence of specialized equipment. Specific antibodies usually appear a week after the onset of symptoms and signs. This should kept in mind when a negative serological test result is being interpreted.

Drug resistance

There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs. Resistance to the fluoroquinolones may be total or partial. The so-called nalidixic-acid-resistant S. typhi (NARST) is a marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. Nalidixic acid itself is never used for the treatment of typhoid. These isolates are susceptible to fluoroquinolones in disc sensitivity testing according to current guidelines. However, the clinical response to treatment

with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-senstive strains. There is a significant number of MDR strains from the Indian subcontinent and some other Asian countries (not Indonesia). S. typhi has recently emerged as a problem in Kenya. Nalidixic-acid-resistant strains are now endemic in many areas of Viet Nam and have also been reported from the Indian subcontinent and Tajikistan. There are disturbing recent reports of the emergence of fluorquinolone-resistant isolates in various parts of Asia and there have been a few reports of resistance to third-generation cephalopsorins in the same region. Reassuringly, however, many of these reports are coupled with evidence of the re-emergence of sensitive isolates in the same regions.

Treatment of typhoid fever

General management

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-up for complications or failure to respond to therapy. However, patients with persistent vomiting, severe diarrhoea and abdominal distension may require hospitalization and parenteral antibiotic therapy.

Antimicrobial therapy

Efficacy, availability and cost are important criteria for the selection of first-line antibiotics to be used in developing countries

The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever

in adults [15]. Evidence from various settings in Asia indicates that the fluoroquinolones are equally effective in the treatment of typhoid fever in children

However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. Table 1 outlines the treatment strategies for uncomplicated typhoid.

Treatment of uncomplicated typhoid fever

	Optimal therapy			**Alternative effective drugs**		
Susceptibility	**Antibiotic**	**Daily dose mg/kg**	**Days**	**Antibiotic**	**Daily dose mg/kg**	**Days**
Fully sensitive	Fluoroquinolone e.g. ofloxacin or ciprofloxacin	15	5-7[a]	Chloramphenicol Amoxicillin	50-75 75-100	14-21 14
Multidrug resistance	Fluoroquinolone or cefixime	15 15-20	5-7 7-14	Azithromycin Cefixime	8-10 15-20	7 7-14
Quinolone resistance[b]	Azithromycin or ceftriaxone	8-10 75	7 10-14	Cefixime	20	7-14

[a] Three-day courses are also effective and are particularly so in epidemic containment.

[b] The optimum treatment for quinolone-resistant typhoid fever has not been determined. Azithromycin, the third-generation cephalosporins, or a 10-14 day course of high-dose

fluoroquinolones, is effective. Combinations of these are now being evaluated.

The available fluoroquinolones (ofloxacin, ciprofloxacin, fleroxacin, pefloxacin) are highly active and equivalent in efficacy (with the exception of norfloxacin which has inadequate oral bioavailability and should not be used in typhoid fever).

The fluoroquinolone drugs are generally very well tolerated. However, in some countries the use of fluoroquinolones is relatively contraindicated in children because of concerns that they may cause articular damage. These agents are not registered for routine use in children. The concerns have arisen because of evidence of articular damage in growing, weight-bearing joints in beagles [16]. There is now extensive experience in the use of these drugs in large numbers of children with a variety of conditions, often with long-term follow-up (cystic fibrosis, typhoid) and in the extensive use of short courses of fluoroquinolones in children for the treatment of both typhoid fever and bacillary dysentery [17]. Their considerable benefits, particularly in areas where there are no affordable oral alternatives, outweigh the putative risk. The only known articular side-effect is Achilles tendon rupture in patients who are also taking corticosteroids, and this has been reported only rarely.

Ciprofloxacin, ofloxacin, pefloxacin and fleroxacin have generally proved effective. There is no evidence of the superiority of any particular fluoroquinolone. Nalidixic acid and norfloxacin do not achieve adequate blood concentrations after oral administration and should not be used.

Chloramphenicol, despite the risk of agranulocytosis in 1 per 10000 patients, is still widely prescribed in developing countries for the treatment of typhoid fever. The disadvantages

of using chloramphenicol include a relatively high rate of relapse (5-7%), long treatment courses (14 days) and the frequent development of a carrier state in adults. The recommended dosage is 50-75 mg per kg per day for 14 days divided into four doses per day, or for at least five to seven days after defervescence. The usual adult dose is 500 mg given four times a day. Oral administration gives slightly greater bioavailability than intramuscular (i.m.) or intravenous (i.v.) administration of the succinate salt.

Ampicillin and amoxicillin are used at 50 to 100 mg per kg per day orally, i.m. or i.v., divided into three or four doses. No benefit has been reported to result from the addition of clavulanic acid to amoxicillin.

Trimethoprim-sulfamethoxazole, (TMP-SMZ) can be used orally or i.v. in adults at a dose of 160 mg TMP plus 800 mg SMZ twice daily or in children at 4 mg TMP per kg and 20 mg SMZ per kg for 14 days.

Of the third-generation cephalosporins, oral cefixime (15-20 mg per kg per day for adults, 100-200 mg twice daily) has been widely used in children in a variety of geographical settings and found to be satisfactory.

Recent data on the use of azithromycin in children indicate that it may be safely given as an alternative agent for the treatment of uncomplicated typhoid fever. Azithromycin in a dose of 500 mg (10 mg/kg) given once daily for seven days has proved effective in the treatment of typhoid fever in adults and children with defervescence times similar to those reported for chloramphenicol. A dose of 1 g per day for five days was also effective in adults [15].

If intravenous antibiotics are required, i.v. cephalosporins can be given in the following doses: ceftriaxone, 50-75 mg per kg per day (2-4 g per day for adults) in one or two doses; cefotaxime, 40-80 mg per kg per day (2-4 g per day for adults) in two or three doses; and cefoperazone 50-100 mg per kg per day (2-4 g per day for adults) in two doses. Ciprofloxacin, ofloxacin and pefloxacin are also available for i.v. use.

Most of the data from randomized controlled trials relate to patients treated in regions of endemicity. Knowledge of the antibiotic sensitivity of the infecting strain is crucial in determining drug choice. If no culture is available, knowledge of likely sensitivity as indicated by the available global data may be useful.

The evidence suggests that the fluoroquinolones are the optimal choice for the treatment of typhoid fever in adults and that they may also be used in children. The recent emergence of resistance to fluoroquinolones, however, suggests that their widespread and indiscriminate use in primary care settings should be restricted. In areas of the world where the fluoroquinolones are not available or not registered for public health use and where the bacterium is still fully sensitive to traditonal first-line drugs (chloramphenicol, amoxicillin or trimethoprim-sulfamethoxazole) these remain appropriate for the treatment of typhoid fever. They are inexpensive, widely available and rarely associated with side-effects.

Management of complications

Both outpatients and inpatients with typhoid fever should be closely monitored for the development of complications. Timely intervention can prevent or reduce morbidity and mortality. The parenteral fluoroquinolones are probably the antibiotics of choice for severe

infections but there have been no randomized antibiotic trials. In severe typhoid the fluoroquinolones are given for a minimum of 10 days. Typhoid fever patients with changes in mental status, characterized by delirium, obtundation and stupor, should be immediately evaluated for meningitis by examination of the cerebrospinal fluid. If the findings are normal and typhoid meningitis is suspected, adults and children should immediately be treated with high-dose intravenous dexamethasone in addition to antimicrobials [18]. If dexamethasone is given in an initial dose of 3 mg/kg by slow i.v. infusion over 30 minutes and if, after six hours, 1 mg/kg is administered and subsequently repeated at six-hourly intervals on seven further occasions, mortality can be reduced by some 80-90% in these high-risk patients. Hydrocortisone in a lower dose is not effective. High-dose steroid treatment can be given before the results of typhoid blood cultures are available if other causes of severe disease are unlikely.

Treatment of severe typhoid fever

	Optimal parenteral drug			**Alternative effective parenteral drug**		
Susceptibility	**Antibiotic**	**Daily dose mg/kg**	**Days**	**Antibiotic**	**Daily dose mg/kg**	**Days**
Fully sensitive	Fluoroquinolone e.g. ofloxacin	15	10-14	Chloramphenicol Amoxicillin TMP-SMX	100 100 8-40	14-21 14 14

Multidrug resistant	Fluoroquinolone	15	10-14	Ceftriaxone or cefotaxime	60 80	10-14
Quinolone resistant	Ceftriaxone or cefotaxime	60 80	10-14	Fluoroquinolone	20	7-14

Patients with intestinal haemorrhage need intensive care, monitoring and blood transfusion. Intervention is not needed unless there is significant blood loss. Surgical consultation for suspected intestinal perforation is indicated. If perforation is confirmed, surgical repair should not be delayed longer than six hours. Metronidazole and gentamicin or ceftriazone should be administered before and after surgery if a fluoroquinolone is not being used to treat leakage of intestinal bacteria into the abdominal cavity. Early intervention is crucial and mortality rates increase as the delay between perforation and surgery lengthens. Mortality rates vary between 10% and 32% [19].

Relapses involving acute illness occur in 5-20% of typhoid fever cases that have apparently been treated successfully. A relapse is heralded by the return of fever soon after the completion of antibiotic treatment. The clinical manifestation is frequently milder than the initial illness. Cultures should be obtained and standard treatment should be administered. In the event of a relapse the absence of schistosomiasis should be confirmed.

Management of carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for S. typhi a year following recovery

from acute illness. Overall, some 1-5% of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, patients older than 50 years, and patients with cholelithiasis or schistosomiasis. If cholelithiasis or schistosomiasis is present the patient probably requires cholecystectomy or antiparasitic medication in addition to antibiotics in order to achieve bacteriological cure. In order to eradicate S. typhi carriage, amoxicillin or ampicillin (100 mg per kg per day) plus probenecid (1 g orally or 25 mg per kg for children) or TMP-SMZ (160 to 800 mg twice daily) is administered for six weeks; about 60% of persons treated with either regimen can be expected to have negative cultures on follow-up. Clearance of up to 80% of chronic carriers can be achieved with the administration of 750 mg of ciprofloxacin twice daily for 28 days or 400 mg of norfloxacin. Other quinolone drugs may yield similar results. Carriers should be excluded from any activities involving food preparation and serving, as should convalescent patients and any persons with possible symptoms of typhoid fever. Although it would be difficult for typhoid carriers in developing countries to follow this recommendation, food handlers should not resume their duties until they have had three negative stool cultures at least one month apart.

Vi antibody determination has been used as a screening technique to identify carriers among food handlers and in outbreak investigations. Vi antibodies are very high in chronic S. typhi carriers.

Prevention of typhoid fever

The major routes of transmission of typhoid fever are through drinking water or eating food contaminated with Salmonella typhi. Prevention is based on ensuring access to safe water

and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour change.

Safe water

Typhoid fever is a waterborne disease and the main preventive measure is to ensure access to safe water. The water needs to be of good quality and must be sufficient to supply all the community with enough drinking water as well as for all other domestic purposes such as cooking and washing.

Food safety

Contaminated food is another important vehicle for typhoid fever transmission.

Appropriate food handling and processing is paramount and the following basic hygiene measures must be implemented or reinforced during epidemics:

•washing hands with soap before preparing or eating food;

•avoiding raw food, shellfish, ice;

•eating only cooked and still hot food or re-heating it.

Typhoid can be transmitted by chronic carriers who do not apply satisfactory food-related hygiene practices. These carriers should be excluded from any activities involving food preparation and serving. They should not resume their duties until they have had three negative stool cultures at least one month apart.

Sanitation

Proper sanitation contributes to reducing the risk of transmission of all diarrhoeal

pathogens including Salmonella typhi.

•Appropriate facilities for human waste disposal must be available for all the community. In an emergency, pit latrines can be quickly built.

•Collection and treatment of sewage, especially during the rainy season, must be implemented

•In areas where typhoid fever is known to be present, the use of human excreta as fertilizers must be discouraged.

Health education

Health education is paramount to raise public awareness on all the above mentioned prevention measures. Health education messages for the vulnerable communities need to be adapted to local conditions and translated into local languages. In order to reach communities, all possible means of communication (e.g. media, schools, women's groups, religious groups) must be applied.

Community involvement is the cornerstone of behaviour change with regard to hygiene and for setting up and maintenance of the needed infrastructures.

In health facilities, all staff must be repeatedly educated about the need for :

•excellent personal hygiene at work;

•isolation measures for the patient;

•disinfection measure.

Vaccination

Currently available vaccines

The old parenteral killed whole-cell vaccine was effective but produced strong side-effects because of LPS. Two safe and effective vaccines are now licensed and available. One is based on defined subunit antigens, the other on whole-cell live attenuated bacteria.

The first of these vaccines, containing Vi polysaccharide, is given in a single dose subcutaneous (s.c.) or i.m. Protection begins seven days after injection, maximum protection being reached 28 days after injection when the highest antibody concentration is obtained. The vaccine is approved for persons aged over two years. Revaccination is recommended every three years for travellers.

The live oral vaccine Ty2la is available in enteric-coated capsule or liquid formulation. It should be taken in three doses two days apart on an empty stomach. It elicits protection as from 10-14 days after the third dose. It is approved for use in children aged at least 5 years. Travellers should be revaccinated annually. The protective efficacy of the enteric-coated capsule formulation seven years after the last dose is still 62% in areas where the disease is endemic; the corresponding figure for the liquid formulation is 70%. Herd immunity was clearly demonstrated during field trials in Chile. Antibiotics should be avoided for seven days

before or after the immunization series.

Future vaccines

Vi-rEPA

A new Vi conjugate candidate vaccine bound to non-toxic recombinant Pseudomonas aeruginosa exotoxin A (rEPA) has enhanced immunogenicity in adults and in children aged 5-14 years, and has induced a booster response in children aged 2-4 years.

S. paratyphi A causes the second commonest enteric fever in Asia. The TAB vaccine, composed of inactivated Salmonella, caused a strong side-reaction. A new S. paratyphi A vaccine composed of the surface O-specific polysaccharide conjugated with tetanus toxoid was shown to be safe and immunogenic in Vietnamese adults, 108 teenagers and 110 children aged 2-4 years.

Other candidates

Three live attenuated candidate vaccines are currently being evaluated. Each is administered as a single oral dose. CVD 908-htrA is an S. typhi strain with a mutation deletion in the htrA gene; a derivative strain, CVD 909, was prepared in order to produce Vi antigen according to constitutive expression. The second candidate is an S. typhi Ty2 strain with triple mutation deletion in the cya, crp and cdt genes. The third is a derivative of an S. typhi Ty2 strain with a double mutation deletion in genes phoP and phoQ.

Recommendations on vaccine use

The occurrence of S. typhi strains that are resistant to fluoroquinolones emphasizes the

need to use safe and effective vaccines to prevent typhoid fever. WHO recommends vaccination for people travelling in high-risk areas where the disease is endemic. People living in such areas, people in refugee camps, microbiologists, sewage workers and children should be the target groups for vaccination.

In routine immunization, therefore, the use of the available typhoid vaccines should be considered in areas where typhoid fever is endemic in children aged over two years. Either Vi or Ty21a vaccine should be used. The Vi vaccine is recommended for use in immuno-compromised hosts.

Literature review

Raghu Raman, et al [20] from Banglore reported on the clinical profile and therapy in enteric fever in 1992. 90 consecutive culture positive enteric fever cases were studied. The clinical profile and the resistance patterns are noted. They found MDRST incidence to be 55% and certain clinical features like fever >104^{o} C, toxemia, hepatomegaly, abdominal distension were significantly more in MDR group, though the age and duration of fever at admission were not significantly different between two groups. They

concluded that characteristic clinical features in MDRST infection as seen in this study should be a pointer to the clinician to suspect and choose appropriate therapy.

S.Mishra, et al [21] studied the clinical profile of multidrug resistant typhoid fever cases admitted in a hospital in New Delhi in 1990. Out of 214 cases admitted with clinical diagnosis of enteric fever, 50 were blood culture positive and were included in the study. 39 out of this 50(78%) were MDR. Children <2 years are found exclusively in MDRTF group and complication like typhoid encephalopathy, hepatitis, bronchopneumonia, myocarditis and pleural effusion were some of the serious complications observed exclusively in MDRTF.

65 blood culture proven cases of typhoid fever were studied by A Sharma et al [22] from Rohtak and 64.6% were MDR. In patients with MDR, the age was higher (9.8 vs. 6.8). They found that there was no difference in the clinical features at the onset between the two groups though the incidence of complications such as shock, encephalopathy, myocarditis and gastric hemorrhage were more frequent in MDR group compared to chlormphenicol sensitive group

U.Madhulika, et al [23] reported the antimicrobial susceptibility pattern of S.typhi isolated in Pondicherry between January 2002 to November 2003. Blood culture was done for 1296 patients suspected to have enteric fever and 157 isolates were obtained and included in the study. They used nalidixic acid susceptibility as a screening test for reduces susceptibility to ciprofloxacin. They found a slight decrease in the occurrence of

MDRST compared to an earlier study, and an increase in resistance to fluroquinolones. They concluded that first line antibiotics may still have a role to play in typhoid fever and ceftriaxone emerges as the sole defence against NARST strains

Similar study reporting the sensitively pattern of salmonella serotypes in Northern India between 1997 to 2001 was done by vikas Gowtam et al [24] from Rohtak. Among the 6956 cultures done, they studied 60 randomly isolated strains and found an increase in occurrence of MDRST and a decrease in susceptibility to ciprofloxacin. They found reemergence of chloramphenicol sensitivity at the extent of 90% and they recommended that sensitivity pattern of causative organism must be sought before instituting appropriate therapy to prevent further emergence of drug resistance.

P.B.Koul, et al[25] form New Delhi studied 48 consecutive culture proven typhoid cases, who had not received any antibiotics prior to admission. Seventy two point eight percent of blood culture positive S.typhi were resistant to chloramphenicol. Of the chloramphenicol resistant strains, 48.5% were resistant to cotrimoxazole and 31.4% to cotrimoxazole and amoxicillin. A combination of cephalexin and gentamicin was successfully used in management of these children. Incidence of shock, myocarditis and encephalopathy was higher among MDRST group.

Chandra R, et al[26] from pondicherry studied multidrug resistant enteric fever

cases. A retrospective review of multidrug resistant typhoid fever cases admitted to paediatric ward of JIPMER hospital was done. Prolonged pyrexia, chills and rigors, toxaemia and tender hepatomegaly were striking features of MDRST cases. Positive blood cultures were observed even after weeks of antibiotic therapy, indicating persistent bacteremia. Ciprofloxacin was found to be best for MDRST in terms of rapid response and cost effectiveness. Cefotaxime has moderate efficacy.

Biswal N, et al[27] from Pondicherry studied all cases of enteric fever admitted between 1988 to 1992. They found there was gradual rise in number of admitted cases. CNS complications were noted more during 1991 and 1992.Other complications like myocarditis. GI bleed were noted in increasing number in 1991-92. Multidrug resistant cases were 46.3% in 1991 and 33.5% in 1992. There has been an increase in resistance of S.typhi to commonly used to drugs like ampicillin, chloramphenicol and cotrimoxazole.

Shorey H.S, et al [28] from Bombay studied an outbreak called 'Dombivali fever' started in March 1990 and spread to all over Bombay and adjoining areas. Later, it was proved to be resistant typhoid fever only. A very high isolation rate (215 isolates) in 1990 as compared to be isolates in previous 2 years (i.e. in 1988 and 89) was observed. The incidence MDRST was very high (67.6%) in 1990, as compared to 6.2% in 1988 and 23.3% in 1989. All strains tested in 1990 were sensitive to ciprofloxacin.

Holder K, et al[29] studied 51 strains of s.typhi, isolated during the outbreak of

enteric fever in and around Calcutta, in 1991. Forty strains were multidrug resistant including chloramphenicol and all these strains were senstitive to cephalexin, gentamicin, furazolidone and ciprofloxacin. Widal test was done in all cases, but the result was inconclusive.

Walia M, et al[30] in their retrospective study on current perspectives of enteric fever analyzed blood culture-confirmed cases of enteric fever diagnosed at Safdarjang Hospital, New Delhi, India from January 2001 to December 2003. Of 377 blood culture-positive cases, 80.6% were Salmonella typhi and 19.4% Salmonella paratyphi A; 21.7% were children aged under 5 years and 6.1% were under 2 years. A significant decline in MDRST was observed, from 21.9% in 2001 to 12.4% in 2003 ($p=0.04$). There was a significant increase in nalidixic acid-resistant Salmonella (NARST) from 56.9% in2001 to 88.9% in 2003 ($p=0.0001$). NARST had a significantly longer fever defervescence time (7.7 v/s 4.7 days, $p<0.001$) and hospital stay (12.1 v/s 8.2 days, $p<0.001$), and higher rates of complications (55.5% vs 24.0%, $p=0.014$) and mortality than nalidixic acid-sensitive Salmonella (NASS). The rate of isolation of MDRST was higher in NARST than NASS (18.8% vs 7.3%, $p=0.013$)

Das U, et al[31] studied Multidrug resistant Salmonella typhi in Rourkela, Orissa. Out of 5410 blood samples 715 samples were positive for Salmonella typhi. The number of multidrug resistant strains of Salmonella typhi isolated constituted almost

16.1% of the total isolates. In this study, chloramphenicol sensitivity was found to be quite high (86.5%) and ceftriaxone showed 100% sensitivity. Resistance to ciprofloxacin was found to be 2.5% which is due to direct consequence of indiscriminate use of antibiotics, either singly or in combination.

Kabra SK et al[32] studied Multidrug-resistant typhoid fever in Ahmedabad India. One hundred children (consecutive) with positive blood culture for Salmonella typhi were studied for clinical profile and complications.The common clinical features were fever (100%), vomiting (58%), abdominal pain (48%), cough (22%) and loose stools (14%) and the Widal test was positive in 75% patients. Eighty per cent of the salmonella isolates were resistant to amoxycillin, chloramphenicol and co-trimoxazole drugs, but all were sensitive to ciprofloxacin and ceftriaxone. Forty patients developed complications: encephalopathy (18), malena (12), haematemesis (10), epistaxis (4), hepatitis (4), acalculous cholecystitis (4), bowel perforation (3) and nephritis (2). Complications were more frequent in children with multidrug-resistant typhoid.The final antibiotic required to render the children afebrile included ciprofloxacin (80), ceftriaxone, amoxycillin (4), chloramphenicol (4), amoxycillin and gentamicin (4), amoxycillin with chloramphenicol (2), and furazolidone (2).The defervescence time was least with ceftriaxone and greatest with amoxycillin. All the affected children made a complete recovery.

Kadhiravan T, et al[33] studied the clinical and laboratory features, fever clearance time and complications prospectively in patients with blood culture-proven typhoid fever, treated at a tertiary care hospital in north India, during the period from November 2001 to October 2003. Susceptibility to amoxycillin, co-trimoxazole, chloramphenicol, ciprofloxacin and ceftriaxone were tested by disc diffusion method. During a two-year period, 60 patients (age [mean +/- SD]: 15 +/- 9 years; males: 40 [67%]) were studied. All isolates were sensitive to ciprofloxacin and ceftriaxone by disc diffusion. However, 11 patients had clinical failure of fluoroquinolone therapy. Infections with NARST isolates (47 [78%]) were significantly associated with longer duration of fever at presentation (median [IQR] 10 [7-15] vs. 4 [3-6] days; P = 0.000), higher frequency of hepatomegaly (57% vs. 15%; P = 0.021), higher levels of aspartate aminotransferase (121 [66-235] vs. 73 [44-119] IU/L; P = 0.033), and increased MIC of ciprofloxacin (0.37 +/- 0.21 vs. 0.17 +/- 0.14 microg/mL; P = 0.005), as compared to infections with nalidixic acid-susceptible isolates. All 11 patients with complications were infected with NARST isolates. Total duration of illness was significantly longer in patients who developed complications than in patients who did not (22 [14.8-32] vs. 12 [9.3-20.3] days; P = 0.011). They concluded that typhoid fever caused by NARST infection is associated with poor clinical outcomes, probably due to delay in initiating appropriate antibiotic therapy. Fluoroquinolone breakpoints for S. typhi need to be redefined and fluoroquinolones should no longer be used as first-line therapy, if the prevalence of NARST is high.

Chande C, et al[34] studied antimicrobial resistance pattern of Salmonella Typhi in central India. A total of 54 isolates of Salmonella were recovered from 1468 blood samples of patients suspected to have enteric fever and admitted in the Government Medical College and Hospital, Nagpur. Antimicrobial susceptibility pattern of these isolates was studied by disc diffusion test. Minimum inhibitory concentration of chloramphenicol was determined by agar dilution method. Of 54 isolates of Salmonella, 51(94%) were S. Typhi and 3 (6%) were S. paratyphi A serotype. Multidrug resistance was observed in 12 (22%) strains of S. Typhi. Thirty five (68%) strains were sensitive to chloramphenicol, ampicillin, gentamicin, cotrimoxazole, cefotaxime and ciprofloxacin. Resistance to two antibiotics was observed in 4 (8%) strains. Cefotaxime resistance was observed in one isolate and gentamycin resistance in two, while none of the isolates was found to be ciprofloxacin resistant. They concluded that multidrug resistance in S. Typhi has decreased from that reported in 1991 though there is a still a small percentage of strains which continue to be multidrug resistant.

STUDY JUSTIFICATION:

Enteric fever continues to be a major public health problem despite the development of newer antibacterial drugs. Drug resistance among S.typhi is known from 1950, when chloramphenicol resistance was first reported. However until 1986 enbloc resistance involving all the three first line drugs was limited to anecdotal case reports. The 1990s witnessed a surge in the incidence of multi drug resistant typhoid fever (MDRTF) and studies from India report an incidence between 10-93% [35,36,37,22].

The present problem is the emergence of quinolone resistance in addition to multi drug resistant salmonella typhi (MDRST). Multi Drug Resistance is defined as resistance to all the first line drugs namely ampicillin, chloramphenicol, and cotrimoxazole. Quinolone resistance is determined by sensitivity to nalidixic acid as suggested by WHO and these nalidixic acid resistant Salmonella typhi (NARST) implies Quinolone resistance to the clinician.

In the present scenario of emergence of nalidixic acid (Quinolone) resistant strains in addition to resistance to conventional drugs and wide variation in susceptibility of S.typhi among different geographical area, it is essential to find the resistance patterns in each locality and hospital including the MDR strains and NARST. In addition by comparing the clinical features of MDRTF with sensitive strains, the risk factor for MDR can be identified and this will provide a clinical clue to the presence of MDR and thus aid in formulating appropriate guidelines for therapy of typhoid fever.

AIM

To study

1. The prevalence and pattern of resistance in typhoid fever including MDRST and NARST
2. Clinical presentation of drug resistant typhoid fever and risk factors for multidrug resistance.

METHODOLOGY

STUDY DESIGN: Descriptive study / Case control study

STUDY PLACE: All medical wards of Institute of Child Health and Hospital for Children

STUDY PERIOD: February 2005 to August 2006

TARGET POPULATION: All children admitted with clinical suspicion of typhoid fever.

STUDY POPULATION: All children with fever (38°C and above) for atleast three days, with a laboratory confirmed positive culture of S.typhi.(WHO definition for confirmed cases of typhoid fever) [38].

EXCLUSION CRITERIA: Associated infections (mixed infections) like UTI, malaria, leptospirosis, prior antibiotic usage

SAMPLE SIZE: All those children who satisfy the inclusion criteria with in the study period.

MANEUVRE

Suspected cases of typhoid fever were admitted in our hospital and detailed history was taken including age, sex, duration of fever and associated symptoms. Also history of previous treatment, socio-economic status and typhoid immunization was elicited. Then complete physical examination was performed to assess the general condition of the patient, nutritional status and temperature; and examination of systems

was done. Significant hepatomegaly was considered to be present, only when the liver size is more than 3.5 cms in new born and 2 cms in children.

After that blood was drawn for investigations like Widal test, enteric culture; and also for other investigations to rule out other causes of fever (like smear for malarial parasite, MSAT, non-enteric culture). Urine routine examination and culture was done.

Blood for enteric culture was taken in Brain-Heart infusion (BHI) broth. One ml of blood was taken using sterile syringe / scalp vein set and put into 10ml of BHI broth to give an optimal dilution of 1:10. Since blood contains substances that inhibit the growth of bacilli, it is essential that the broth be taken in sufficient quantity to provide at least four fold dilution of blood. Liquoid (Sodium polyethanol sulphonate) was added to BHI broth during its preparation to counteract the bactericidal action of blood. BHI broth was kept in refrigerator prior to use to avoid bacterial contamination. Before adding blood, the BHI broth was brought down to normal temperature and blood was added. After adding blood, BHI broth was immediately transported to microbiology lab of the hospital. If it was not possible, as in night times, the bottle was kept in the incubator and then transported to lab. This avoided bacterial overgrowth and enhanced the chances of positivity of salmonella typhi growth.

ENTERIC CULTURE

In the lab, BHI broth was incubated at $37^{O}C$ in the incubator for 24hrs. Then subculture was done in MacConkey agar and salmonella shigella agar, which is a special

media for S.typhi. After overnight incubation at 37^{O}C, the pale smooth, nonlactose fermenting colonies were looked for. If present, then battery of tests was done to confirm S.typhi growth. S.typhi has the following characters:

1. Motility by hanging drop: Motile
2. Bio-chemical characters

   S.typhi is citrate positive, indole and urease negative and ferments glucose, mannitol and maltose, not sucrose and lactose.

3. Agglutination

   A loopful of growth from the agar is emulsified with saline and a loopful of typhoid 'H' and 'O' antiserum were added. Presence of agglutination indicates salmonella growth.

After confirming the growth, antibiotic sensitivity was done in Muller-Hinton agar, using Kirby-Baur technique. A loopful of growth was taken from the agar and inoculated in peptone water; after 10 minutes, this peptone water was poured over the Muller-Hinton agar to cover its entire surface; the excess peptone water was poured into the bottle.

Then antibiotic discs like ampicillin, chloramphenicol and ciprofloxacin etc., were plated and incubated for 24 hrs at 37^{O}C. Then zone of inhibition was measured and results were interpreted.

Culture negativity was declared only after seven days. Then the patients were observed daily for clinical improvement in the form of cessation of fever and improvement in the general well being. Also complications (due to disease or drug) were carefully watched for. If the patient becomes culture positive, then the antibiotics were decided according to sensitivity pattern. All the cases were observed daily till the time of discharge.

STATISTICAL ANALYSIS

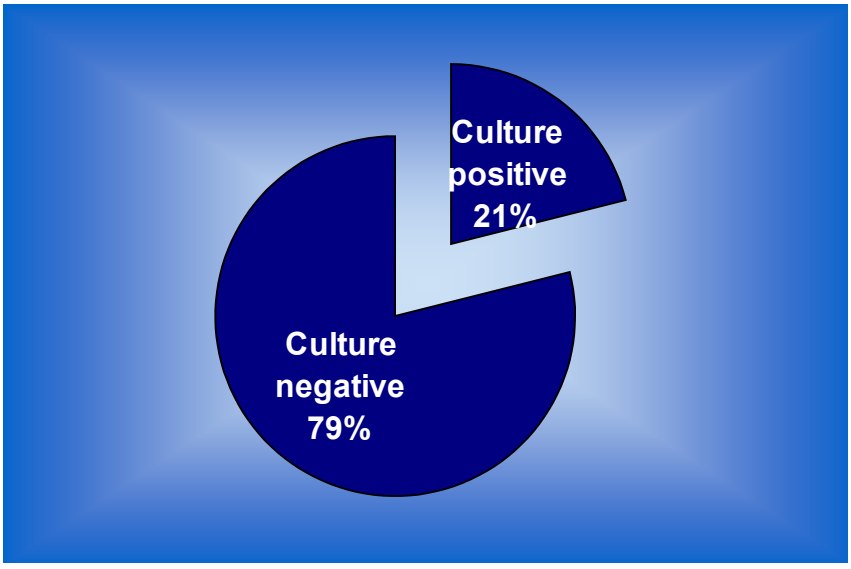
1. Proportion of MDRTF and NARST among culture proven typhoid fever was found.

2. To associate various features to drug resistance, univariate analysis was done to arrive at odds ratio (OR) with 95% confidence interval.

3. To associate how far individual factors contribute independently for drug resistance, multi variate analysis was done.

RESULTS

Out of 186 samples of the enteric culture sent, 39 grew S.typhi with a culture positive rate of 21%. Only those with culture proven typhoid fever were included in the study (WHO definition of confirmed cases of typhoid).



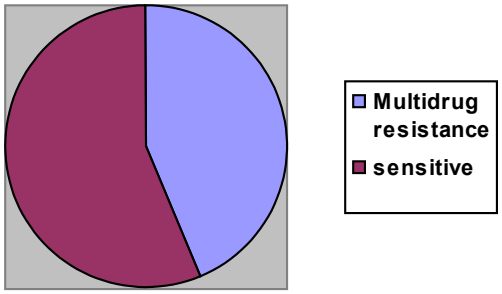
The following is the invitro antibiotic susceptibility pattern of the 39 cultures grown

Tab 1 : Antibiotic susceptibility pattern among 39 culture positive cases

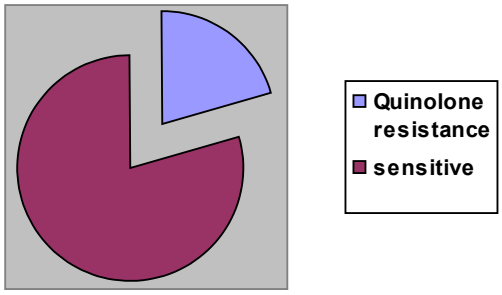
Sl No	Drug	Sensitive		Resistant	
		N =	%	N =	%
1	Ampicillin	16	41	23	59
2	Chloramphenicol	22	56.4	17	43.6
3	Cotrimoxazole	10	25.6	29	74.4
4	Cefotaxime	30	76.9	9	23.1
5	Ceftriaxone	35	89.7	4	10.3
6	Nalidixic acid	31	79.4	8	20.6
7	Ciprofloxacin	31	79.4	8	20.6
8	Amikacin	34	87.2	5	12.8

Multidrug resistance is present when the isolates were resistant to all the three first line drugs namely ampicillin, chloramphenicol and cotrimoxazole. The isolates are considered drug sensitive if they are sensitive to at least one of these first line drugs. The incidence of multi-drug resistance (defined by resistance to all the first line drugs namely ampicillin, cotrimoxazole and chloramphenicol) and quinolone resistance was found to be

Incidence of multidrug resistance: 43.6% (N = 17)



Incidence of quinolone resistance: 20.5% (N = 8)



The following is the demographic profile of the various cases included in the study

Tab 2: demographic profile of the various cases included in the study

	Total (N =39)		MDR (N=17)		Sensitive (N=22)	
	n	%	n	%	n	%
Age						

<5 years	19	48.7	12	70.6	7	31.8
> 5 years	20	51.3	5	29.4	15	68.2
Sex						
Male	22	56.4	10	58.8	12	54.5
Female	17	43.6	7	41.2	10	45.5
Season						
I	21	53.8	9	52.9	12	54.5
II	18	46.2	8	47.1	10	45.5

The following is the symptom profile of the multi-drug resistant and drug sensitive typhoid fever

Tab 3: Clinical profile – symptoms

	Total (N =39)		MDR (N=17)		Sensitive (N=22)		p-value		
	n	%	n	%	n	%		O.R.	95% C.I.

Age <5 years	19	48.7	12	70.6	7	31.8	0.02	5.1	1.1 , 26.3
Fever > 7 days	20	51.3	13	76.5	7	31.8	0.01	7.0	1.4 , 38.8
Chills/Rigor	13	33.3	6	35.3	7	31.8	0.82	1.2	0.2 , 5.5
Abdominal Symptoms	21	53.8	9	52.9	12	54.5	0.9	0.9	0.2 , 4.0
Respiratory Symptoms	20	51.3	12	70.6	8	36.4	0.08	3.3	0.7 , 15.2
CNS Symptoms	4	10.3	2	11.8	2	9.1	0.8	1.3	0.1 , 15.5

The following is the clinical profile - signs of the multi-drug resistant and sensitive typhoid fever

Tab 4: clinical profile – signs

	Total (N =39)		MDR (N=17)		Sensitive (N=22)		p-value	O.R.	95% C.I.
	n	%	n	%	n	%			
Toxic look	11	28.2	6	35.3	5	22.7	0.39	1.9	0.4 , 9.5

Coated tongue	12	30.8	6	35.3	6	27.3	0.59	1.5	0.3 , 7.1
Hepatomegaly	6	15.4	4	23.5	2	9.1	0.22	3.1	0.4 , 28.9
Splenomegaly	17	43.6	1	5.9	16	72.7	0.00	0.02	0.0 , 0.2
Hepatosplenomegaly	16	41.0	12	70.6	4	18.2	0.00	10.8	2.0 , 67.7
Lung signs	9	23.1	4	23.5	5	22.7	0.95	1.1	0.2 , 5.9
Defervescence >5 days	14	35.9	8	47.1	6	27.3	0.20	2.4	0.5 , 11.3
Duration of hospital stay > 5 days	16	41.0	9	52.9	7	31.8	0.18	2.4	0.5 , 11.1

Incidence of complications among the two groups is as follows

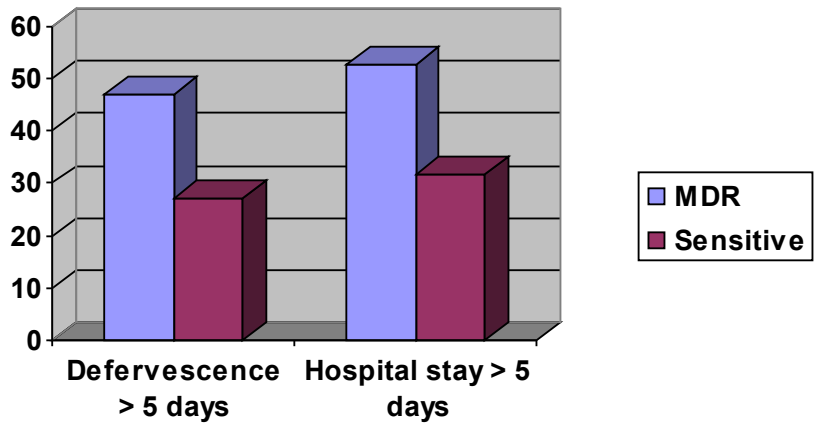
Tab 5: Complications

	Total (N =39)		MDR (N=17)		Sensitive (N=22)		p-value	O.R.	95% C.I.
	n	%	n	%	n	%			
Abnormal LFT	8	20.5	7	41.2	1	4.5	0.00	14.7	1.4 , 364.6
Thrombocytopenia	9	23.1	7	41.2	2	9.1	0.02	7.0	1.0 , 60.6

Shock	2	5.1	2	11.8	-	0.0	0.09	-	-
Gastrointestinal – hemorrhage or perforation	1	2.6	1	5.9	-	0.0	0.25	-	-

There were no cases of clinical jaundice, encephalopathy or mortality.

The results of follow up of these patients were compared between the two groups



Defervescence was taken as the time from admission and commencement of treatment to the time till fever subsided. It was observed that time taken for defervescence and duration of

hospital stay were significantly more in the multidrug resistant group though it did not satisfy the statistical criteria.

The clinical profile of the eight cases of quinolone (Nalidixic acid resistant) Salmonella typhi were as follows

Tab 6: clinical profile of quinolone (Nalidixic acid resistant) Salmonella typhi

Clinical features	Quinolone resistance Total (N =8)	
	n	%
Age <5 years	5	62.5
Fever > 7 days	6	75
Chills/Rigor	2	25
Abdominal Symptoms	3	37.5
Respiratory Symptoms	3	37.5

CNS Symptoms	2	25
Toxic look	3	37.5
Coated tongue	4	50
Hepatomegaly	3	37.5
Splenomegaly	2	25
Hepatosplenomegaly	3	37.5
Lung signs	2	25
Abnormal LFT	3	37.5
Thrombocytopenia	3	37.5

DISCUSSION:

There is a wide variation in the culture positivity rate reported from different studies in India. It is as low as 12% in a recent large study conducted in JIPMER [23,34] to as high as 80% in some studies. The culture positivity rate from the present study is 21%. This is quite an improvement compared to the culture positivity rates from the same institute in the preceeding years.

It was seen that the majority of the strains grown were found to be resistant to cotrimoxazole (74.4%) and ampicillin (59%) and most strains were found to be sensitive to ceftriaxone (89.7%) and amikacin (87.2%). Though amikacin is not considered to be a useful drug against Salmonella typhi, it has shown a very high sensitivity in the invitro susceptability testing. Whether it is really useful in vivo either as monotherapy or as a combination especially in drug resistant cases needs to be determined.

Majority of the strains were also sensitive to quinolones (79.4%) and cefotaxime (76.9%). Though there is a wide variation in chloramphenicol sensitivity reported from the different parts of the country, ranging from 0% sensitivity to 90% sensitivity [24,25], in the present study it is observed that chloramphenicol is sensitive in 56.4%. More importantly, chloramphenicol has been found to be quite sensitive in some of the quinolone resistant and cephalosporin resistant isolates. Thus, as reported in some of the studies, chloramphenicol sensitivity may be re-emerging and there is a constant need to monitor the sensitivity pattern of S.typhi isolates.

Among the 39 cultures grown, the incidence of multi-drug resistance was found to be 43.7% (n = 17). Among the 17 multi-drug resistant strains in the present study, 15 were sensitive to ceftriaxone and 14 were sensitive to quinolone. Comparing other studies from India, the incidence of multi-drug resistant typhoid fever varied from 10% to 93% [20,21,22,23,27,28,29].

The incidence of quinolone resistance in the present study was found to be 20.5% (n = 8). Though there are number of studies from India reporting the incidence of multi-drug resistant typhoid fever, the data on quinolone resistance is very limited [23,24,30,33]. Among the eight quinolone resistant cases in the present study, six were sensitive to ceftriaxone and five were sensitive to chloramphenicol. Among the two cases which were quinolone and ceftriaxone resistant, one was sensitive to chloramphenicol and the other resistant to all the other drugs, except amikacin and ceftazidime.

CLINICAL PROFILE

Age: The mean age of the patients among the entire study group was 5.4 ± 2.6 years, while the mean age in the drug resistant group and the drug sensitive group were 4.0 ± 2.1 and 6.4 ± 2.5 respectively. Though the total number of children < 5 years was 19 in the overall study group, majority (70.6%, n = 12) were in the drug resistant group and only 31.8% (n = 7) were in the drug sensitive group (P = 0.02).

Sex: There were totally twenty two males and seventeen females in the study group. There was

no difference in the sex distribution among drug resistant and sensitive groups.

There were no seasonal variation and cases occurred throughout the year indicating that typhoid fever is endemic in this region.

The mean duration of fever in the whole group was 6.9 ± 2.4 days, while in the drug resistant group it was 8.5 ± 1.9 days and it was 5.7 ± 2.0 in the drug sensitive group ($P = 0.001$). Though fever was the presenting problem in all the children, prolonged fever > 7 days was found in significantly major proportion in the drug resistant group (76.5%, n = 13) than in the drug sensitive group (31.8%, n = 7). Thus prolonged fever > 7 days was identified to be one of the important risk factor for drug resistance.

Though respiratory symptoms like running nose, sneezing, cough etc were found more in the drug resistant group (70.6% vs 36.4%), it did not meet the required statistical criteria ($P > 0.05$). Chills and rigor was found in one-third, abdominal symptoms like nausea, vomiting and loose stools were found in nearly half of the patients and central nervous symptoms like head-ache and letharginess were found in nearly 10% of the patients, without any difference between the two groups. There were no cases of profound loss of consciousness or seizures.

Toxic look was found in 11 out of the 39 patients and coated tongue was found in 12 patients without much difference between the two groups.

Isolated hepatomegaly was found in six out of the 39 children, four in the multi-drug resistant group and two in the drug sensitive group. Whereas isolated hepatomegaly was found only in few patients, isolated splenomegaly was found almost exclusive in the drug sensitive group with only one patient from drug resistant group had isolated splenomegaly. Hepatosplenomegaly was found in significantly major proportion in drug resistant group (70.6%, n =12), while in the drug sensitive group it was only 18.2% (n = 4) ($P < 0.001$). It is also interesting to note that all the patients had one or other form of organomegaly, with isolated splenomegaly predominating in the drug sensitive group and hepatosplenomegaly in the resistant group. Lung signs occurred in almost one-fourth of the patients, though equally in both the groups.

COMPLICATIONS:

Though higher complication rates have been observed in some of the studies in India [21,22,32], complications are few in the present study. Abnormal liver function test and thrombocytopenia were the two major complications observed, primarily in the multi-drug resistant group and both assuming a statistically significance. Though no cases of overt clinical jaundice was observed, abnormal liver function in the form of mild elevation of bilirubin or more than twice the normal level of liver enzymes were observed in eight cases, seven in the multi-drug resistant group (41.2%) and one from sensitive group (4.5%) ($P <0.01$).

Similarly thrombocytopenia, defined by platelet count <100,000, were found in nine patients, significantly more in the drug resistant group (41.2%, n = 7), while two patients from drug sensitive group (9.1%) also had thrombocytopenia. Except for the one patient, which presented with malena, none of the other had any clinical bleeding.

Two of the patients, both from the multi-drug resistant group, had shock at presentation. Both the children were corrected with two boluses of isotonic fluids. The exact reason for shock, whether it is fluid leak into third space, inadequate intake of oral fluids or increased loss of fluids secondary to high grade fever or combination of these, were not known. But both the children recovered quickly without any other complications .

Only one patient from the multi-drug resistant group had gastro-intestinal hemorrhage in the form of malena. The child also had a lowest platelet count of 85,000. The reason for the hemorrhage, whether due to thrombocytopenia or intestinal foci of salmonella was not known. The child had fever lasting for more than six days after admission, but recovered subsequently.

There were no cases of encephalopathy or other complications and there were no deaths.

FOLLOW UP :

The mean duration for defervescence was 4.1 ± 2.2 days in the overall study group, whereas it was 5.4 ± 1.8 in the drug resistant group and 3.1 ± 2.0 in the drug sensitive group. Thus there is a statistically significant prolonged duration for defervescence in the multi-drug resistant group ($P = 0.004$). Similarly, the duration of hospital stay was significantly more in the multi-drug resistant group (6.5 ± 1.8 vs 4.9 ± 1.7) ($P = 0.01$).

Thus, in the present study, it is found by univariate ordinal regression that the following are the

clinical pointers to multi-drug resistance. These include

1. Age < 5 years
2. Prolonged fever > 7 days
3. Hepatosplenomegaly
4. Abnormal liver function test
5. Thrombocytopenia

Tab 7: clinical pointers to multi-drug resistance

	Total (N =39)		MDR (N=17)		Sensitive (N=22)		p-value	O.R.	95% C.I.
	n	%	n	%	n	%			
Age <5 years	19	48.7	12	70.6	7	31.8	0.02	5.1	1.1 , 26.3
Fever > 7 days	20	51.3	13	76.5	7	31.8	0.01	7.0	1.4 , 38.8
Hepatosplenomegaly	16	41.0	12	70.6	4	18.2	0.00	10.8	2.0 , 67.7
Abnormal LFT	8	20.5	7	41.2	1	4.5	0.00	14.7	1.4 , 364.6
Thrombocytopenia	9	23.1	7	41.2	2	9.1	0.02	7.0	1.0 , 60.6

And by multiple logistic regressions it is found that fever { p = 0.007 [O. R. = 1.8] and 95 % C.I. [1.2, 2.9] } and hepatosplenomegaly { p = 0.03 [O. R. = 6.9] and 95 % C.I. [1.2, 38.9] } are independent risk factors for drug resistance.

Hence these risk factors might aid the physicians to start appropriate antibiotics as per the prevailing antibiotic susceptibility pattern in the locality to prevent major complications.

Quinolone resistance:

Among the eight cases found to have quinolone resistance, five were boys and five were younger than five years (62.5 %). Similar to the multidrug resistant typhoid fever, prolonged fever of more than seven days were present significantly more in quinolone resistant group (n = 6, 75%). Abdominal symptoms, respiratory symptoms, toxic look and coated tongue were present in one third to half of these patients. While isolated splenomegaly was distinctly present in the drug sensitive typhoid fever and hepatosplenomegaly in the multidrug resistant group, no such difference in organomegaly was present in quinolone resistant group with three children had hepatomegaly, three had both hepatosplenomegaly and two had isolated splenomegaly. While abnormal liver function test and thrombocytopenia were observed in three of the eight children, other complications like shock, encephalopathy, gastrointestinal hemorrhage or mortality were not seen in the quinolone resistant group.

CONCLUSION

1. Incidence of multidrug resistant and quinolone resistant typhoid fever was found to be 43.6% and 20.5% respectively which vary with time and place. Hence there is a constant need to monitor antibiotic sensitivity pattern of S.typhi and periodically review the antibiotic policy in the hospital and the community so as to effectively utilize these antibiotics.
2. Majority of S.typhi isolates were found to be resistant to cotrimoxazole (74.4 %) and ampicillin (59 %) and most strains were observed to be sensitive to ceftriaxone (89.7%) and amikacin (87.2%).
3. Though amikacin was found to be sensitive in as high as 87.2%, its usefulness in vivo need to assessed.
4. Chloramphenicol sensitivity was observed among 56.4% of the isolates. More importantly some of the quinolone resistant and ceftriaxone resistant strains were found to be sensitive to chloramphenicol.
5. Age less than five years, prolonged fever for more than seven days, hepatosplenomegaly, abnormal liver function test and thrombocytopenia are specific risk factors for multidrug resistant typhoid fever and need early and aggressive management for prevention of complications.
6. Quinolone resistance may be a problem in future in typhoid fever and specific risk factors and alternative therapeutic strategies need to be evaluated.

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ANNEXURE

PROFORMA

S.No:

Name: Age: Sex:

I.P.No: DOA:

Address:

Clinical Features:

H/O

Fever:

Duration:

Pattern:

Associated chills/rigor:

Bowel habits(Constipation / Diarrhea):

Vomiting:

Abdominal pain:

Abdominal distension:

Cough:

Anorexia:

Joint pain:

Myalgia:

Epistaxis:

Headache:

Alteration in mental status:

Seizures:

Loss of consciousness:

H/o contact with known typhoid fever:

Prior treatment H/O:

Immunization H/O:

Vital Signs:

PR:

BP:

RR:

Temperature:

General Examination:

Anemia:

Jaundice:

Edema:

Lymphadenopathy:

Toxic Look:

Rose Spot:

Coated Tongue:

Others:

Systemic Examination:

Abdomen:

Respiratory System:

Cardio Vascular System:

Central Nervous System:

Musculoskeletal:

Investigations:

CBC: Urine R/E:

C & S :

PS-MP: CXR :

LFT: Mantoux:

CSF analysis:

Blood culture:

Organism:

Sensitivity pattern:

Stool culture:

Others:

Treatment:

Follow up :

Features	D1	D2	D3	D4	D5	D6	D7
Fever							
Organomegaly							
Complications							
Hospital stay							